

WHAT IS CLAIMED IS:

1. A method for quantifying the expression of target gene sequences of interest in a sample, comprising the steps of:

- 5 (i) amplifying one or more cDNA molecules derived from a sample by polymerase chain reaction in the presence of a plurality of amplification primer sets suitable for amplifying target gene sequence of interest, and in the presence of at least one oligonucleotide probe complementary to a region of an amplified target gene sequence, said at least one oligonucleotide probe optionally labeled with a labeling
10 system suitable for monitoring the amplification reaction as a function of time, and (ii) quantifying the target gene sequences amplified in step (i).

2. The method of Claim 1 in which the amplification of step (i) is further carried out in the presence of a reverse transcriptase such that the polymerase chain reaction is reverse-transcription polymerase chain reaction and wherein the one or
15 more cDNA molecules is obtained from mRNA derived from the sample.

3. The method of Claim 1 in which the one or more cDNA molecules comprise a cDNA library.

4. The method of Claim 1 in which said quantifying comprises analysis by a method selected from at least one of the group consisting of real-time polymerase
20 chain reaction amplification, DNA microarray hybridization analysis, electrophoresis and chromatography.

5. The method of Claim 1 in which the polymerase chain reaction of step (i) is carried out for a number of cycles such that the amplification remains in the linear range.

25 6. The method of Claim 1 in which the amplification in step (i) is achieved with a thermostable DNA polymerase.

7. The method of Claim 1 in which said at least one oligonucleotide probe is labeled with a moiety capable of producing a detectable signal.

8. The method of Claim 7 in which the label is a fluorophore.

9. The method of Claim 7 in which said at least one oligonucleotide probe is selected from the group consisting of 5'-exonuclease probes, stem-loop beacon probes and stemless beacon probes.

10. The method of Claim 1 in which said at least one oligonucleotide probe
5 comprises a plurality of oligonucleotide probes, each of which is complementary to a region of a different amplified target gene sequence of interest.

11. The method of Claim 10 in which the product of step (i) is divided into a plurality of aliquots and said quantifying in step (ii) is performed on said aliquots.

12. The method of Claim 11 wherein the number of aliquots is equal to the
10 number of primer pairs used in the multiplex amplification.

13. The method of Claim 12 in which step (ii) comprises amplifying the product in each aliquot by polymerase chain reaction in the presence of an amplification primer set suitable for amplifying one of the target sequences of the plurality.

14. The method of Claim 13 in which the amplifying in step (ii) is further
15 carried out in the presence of an oligonucleotide probe complementary to a region of a different amplified target gene sequence of interest, wherein each probe in step (ii) comprises one of the oligonucleotide probes in step (i).

15. The method of Claim 12 in which the sequences of the amplification
20 primer sets of step (i) are the same as the sequences of the amplification primer sets of step (ii).

16. The method of Claim 11 in which the amplifying in step (ii) is further
carried out in the presence of a molecule that produces a detectable signal when bound to a double-stranded polynucleotide suitable for monitoring the amplification reaction
25 as a function of time.

17. The method of Claim 16 in which the molecule is selected from the group consisting of an intercalating dye and a minor groove binding dye.

18. The method of Claim 17 in which the molecule is selected from the group consisting of SYBR® green I and ethidium bromide.

19. A method for determining a gene expression profile in a sample,
comprising the steps of:

(i) amplifying one or more cDNA molecules derived from said sample by
polymerase chain reaction in the presence of a plurality of amplification primer sets
5 suitable for amplifying target gene sequences of interest;

(ii) identifying amplified target gene sequences having an observed efficiency
of amplification greater than a selected level; and

(iii) quantifying the target gene sequences identified in step (ii) to obtain a gene
expression profile.

10 20. The method of Claim 19 in which the amplification of step (i) is further
carried out in the presence of a reverse transcriptase such that the polymerase chain
reaction is reverse-transcription polymerase chain reaction and wherein the one or
more cDNA molecules is obtained from mRNA derived from the sample.

21. The method of Claim 19 in which the one or more cDNA molecules
15 comprise a cDNA library.

22. The method of Claim 19 in which said selected level is 70%.

23. The method of Claim 19 in which said selected level is 90%.

24. The method of Claim 19 in which said quantifying comprises analysis
by a method selected from at least one of the group consisting of real-time polymerase
20 chain reaction amplification, DNA microarray hybridization analysis, electrophoresis
and chromatography.

25. The method of Claim 19 in which the amplifying in step (i) is further
carried out in the presence of an oligonucleotide probe complementary to a region of
an amplified target gene sequence of interest, said probe being labeled with a labeling
25 system suitable for monitoring the amplification reaction in step (i) as a function of
time.

26. The method of Claim 19 in which the product of step (i) is divided into
a plurality of aliquots and said quantifying in step (ii) is performed on said aliquots.

27. The method of Claim 26 in which step (ii) comprises amplifying the
30 product in one or more separate aliquots by polymerase chain reaction in the presence

of an amplification primer set suitable for amplifying one of the target sequences of the plurality.

28. The method of Claim 27 in which the sequences of the amplification primer sets of step (i) are the same as the sequences of the amplification primer sets of step (ii).

29. The method of Claim 27 in which the amplifying in step (ii) is further carried out in the presence of a molecule that produces a detectable signal when bound to a double-stranded polynucleotide suitable for monitoring the amplification reaction as a function of time.

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30. The method of Claim 29 in which the molecule is selected from the group consisting of an intercalating dye and a minor groove binding dye.

31. The method of Claim 30 in which the molecule is selected from the group consisting of SYBR® green I and ethidium bromide.

32. The method of Claim 27 in which the polymerase chain reaction of step (i) is carried out for a number of cycles such that the amplification remains in the linear range.

33. A method of generating a plurality of target sequences of interest, comprising the step of:
amplifying by polymerase chain reaction one or more target polynucleotides in the presence of a plurality of amplification primers suitable for amplifying target sequences of interest and in the presence of at least one oligonucleotide probe complementary to a region of an amplified target sequence of interest, said oligonucleotide probe being optionally labeled with a labeling system suitable for monitoring an amplification reaction as a function of time.

34. The method of Claim 33 in which said at least one oligonucleotide probe comprises a plurality of oligonucleotide probes, each of which is complementary to a region of an amplified target sequence of interest.

35. The method of Claim 33 in which the product of the amplification is further subjected to at least one assay selected from the group consisting of single polynucleotide polymorphism analysis, genotyping analysis, gene expression analysis,

fingerprinting analysis, analysis of gene mutations for genetic diagnoses, analysis of rare expressed genes in cells, nucleic acid sequencing, nucleic acid mini-sequencing and gene expression analysis.

36. The method of Claim 33 in which the product of the amplification is further subjected to at least one assay selected from the group consisting of chromatography, electrophoresis, and staining with a dye or hybridization probe.

37. The method of Claim 33 in which the product of the amplification is divided into a plurality of aliquots.

38. The method of Claim 35 in which the product of the amplification is divided into a plurality of aliquots and wherein said at least one assay is performed on at least one of said aliquots.

39. The method of Claim 38 wherein the number of aliquots is equal to the number of primer pairs used in said amplifying.

40. A method of generating a plurality of different target sequences of interest, comprising the step of:
amplifying by polymerase chain reaction one or more target polynucleotides in the presence of a plurality of amplification primers suitable for amplifying target sequences of interest and in the presence of a molecule that produces a detectable signal when bound to a double-stranded polynucleotide, said molecule suitable for monitoring the amplification reaction as a function of time, thereby generating a plurality of target sequences of interest.

41. The method of Claim 40 in which the molecule is selected from the group consisting of an intercalating dye and a minor groove binding dye.

42. The method of Claim 41 in which the molecule is selected from the group consisting of SYBR® green I and ethidium bromide.

43. The method as in any one of claims 1, 19, 33 and 40 in which the amplification is carried out in the presence of uracil N-glycosylase.